Commercializing Unique Nanoparticle-Imaging-Delivery Platform for Enhanced Cancer Therapy

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ABSTRACT

We have developed a versatile NID™ (Nanoparticle-Imaging-Delivdry) platform that enables utilizing precisely engineered nanoparticle properties for specfic biomedical applications. The NID™ platform generates nanoparticles with optimal size, shape, surface, magnetic and optical properties. Our nanoparticles demonstrate enhanced properties for cancer diagnosis and treatment applications. Examples are provided here for cell separation utilizing magnetic and fluorescent magnetic nanoparticles that could be applied for fast identification of circulating tumor cells, and for cancer drug delivery utilizing multifunctional nanoparticles with extremely enhanced permeability and retention effect, >30 day cancer region retention after single dose intraveneous injection, controllable pharmacokinetics, all FDA approvable component materials, and superior magnetic, fluorescent and cancer targeting proerties.

Keywords: nanoparticle imaging delivery platform, magnetic nanoparticles, fluorescent magnetic nanoparticles, cancer drug delivery, magnetic cell separation.

INTRODUCTION

Nanoparticles have been studied extensively for their biomedical applications over the last two decades. The importance of this burgeoning field stems from the fundamental properties of materials at the nanoscale that are governed by fundamental concepts in condensed matter physics. [1] For biological and medical applications of nanoparticles, there are three important aspects worthy to note: 1. the unique properties of nanoparticles that do not exist in bulk materials, 2. the nanometer size scale that is also the length scale of fundamental biological components such as proteins and nucleic acids, and 3. the largely increased surface area to volume ratio that not only affects physical properties of nanoparticles such as surface energy and melting temperature, but also provdes a rich decorative interface between nanoparticles and biosystem. Widely used nanomaterials in biology include superparamagnetic iron oxide nanoparticles, semiconductoring quantum dots, and metallic gold nanoparticles. All of these are beautiful domonstrations of the uniqueness of nanoparticles with inorganic component material. Superparamagnetism occurs when the size scale of iron oxide is reduced to below ~20 nm, when thermal fluctuation could overcome magentic anisotrophy, resulting in the widely used superparamagnetic property such that the nanoparticle shows magentic response under a magnetic field but behaves as non-magnetic material when the magnetic field is removed. For semiconducting quantum dots, the size dependent fluorscence that covers the whole range of the rainbow color results from the "quantum confinement" where the electronic energy levels could be precisely tuned by controlling the sizes of nanoparticles, at the scale smaller than ~ 10nm. Surface plasmon resonance of gold nanoparticles origins from the interaction of light with electrons on gold nanoparticle surfaces. The collective osicilations of valence electrons of gold nanoaprticles under the resonance wavelength results in strong extinction of light. The red color in routine paper strip immunoassay testing of many diagnostic labs is from gold nanoparticles due to the surface plasmon resonance effect.

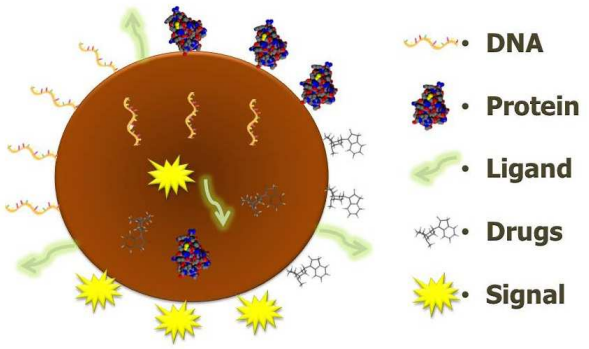

Figure 1. A cartoon to illustrate versatility of the NID™ platform. Different molecules, such as DNA, protein, binding ligand, drugs or signal generating molecules could be incorporated into the nanoparticles for a variety of research, diagnostic, and therapeutic applications.

Our NID™ (Nanoparticle-Imaging-Delivdry) platform takes advantage of these unique properties of inorganic component material, for which they are used not only as the carrier, but also for the additonal functionalities based on their unique physical properties, for example, magnetic separation and capturing, magnetic resonance and/or optical imaging. Such platform distinguishes itself from the pure organic based nanoparticle formulations, such as albumin nanoparticles, PLA, PLGA, or dextran based polymeric nanoparticles, and lyposomes, for which the nanoparticle's function purely rely on its nanosize scale without the existence of additional physical properties.

The versatility and flexibility in combining materials with different properties into the NID™ platform makes it a powerful tool to generate multifunctional nanoparticles for enhanced cancer diagnostic and therapeutic applications.

One of our recent work illustrated such comcept with experimental demonstrations in living subjects. [2] In this paper, we will focus on two applications aimed to further promote the use of the NID™ platform for enhanced cancer diagnosis and therapy: 1. highly specific magentic and fluorescent magnetic nanoparticles for circulating tumor cell separation and identification; 2. multifunctioal nanoparticles for enhanced cancer imaging, targeting and drug delivvery.

CANCER CELL ISOLATION AND IDENTIFICATION WITH MAGNETIC AND FLUORESCENT MAGNETIC NANOPARTICLES

Cell isolation and identification are widely needed in biomedical research and clinical diagnostics/therapeutics. Due to the low frequency of rare cell population, cell separation and quantification could be very challenging under certain circumstances, for example, the isolation of circulating tumor cells (CTCs) from whole blood samples requires the capture sensitivity of one CTC in one billion background blood cells. This is extremely challenging and prevented CTC isolation using common cell sorting techniques like flow cytometer, which has a low sensitivity and involves higher cost of the facility. [3] Magnetic separation provides a fast, simple, gentle and cost-effective way to isolate rare cells from whole blood samples.

Magnetic nanoparticles generated through our NID™ platform present optimal surface chemistry for specific cell isolation. For a variety of cancer cell lines in mixed cell population, and for cancer cells spiked in whole blood samples, the isolated cell population demonstrates extremely high purity. Figure 2 shows the experimental results of a few examples in utilizing the NID™ platform generated nanoparticles for cell separation. The experimental scheme utilizes the specific binding between an antibody and its cell surface marker antigen. To keep the experimental scheme simple, streptavidin coated magnetic nanoparticles (MagVigen™-streptavidin) were used, and the antibody is biotinylated. Since our magnetic nanoparticle beads have strong magnetic moment to directly respond to the magnetic attraction from a common external magnet, it is extremely easy to purify the product after beads based reaction. This allows us to set up a general protocol where biotinylated antibodies will react with MagVigen™-streptavidin first, then following magnetic purification, the MagVigen™-antibody will be incubated with cell samples for isolation of the specific cell population. In Figure 2A-C, we tested for cell separation of a particular cell population in a mixture of two cell lines. Human prostate adenocarcinoma cells LNCaP and human B lymphoma cells Oc1-ly8 were premixed with a total number of cells around 1 million. LNCaP express surface EpCAM, the cancer-associated epithelial cell adhesion molecule that is often applied in the selection of circulating tumor cells from whole blood samples. Here LNCaP were selected as the targeting cells for selection. Oc1-ly8 cells don't have surface EpCAM and were used as background interfering cells. Before cell mixing, Oc1-ly8 cells were pre-stained with a green fluorescence from CFSE for cell differentiation and counting. A general image showing the mixture of LNCaP and Oc1-ly8 was displayed in Figure 2a. High cell purity after magnetic separation were manifested across a wide range of the percentage ratio of targeted cells in the mixture, from around 5% to 65%, for both when the number of targeted cell LNCaP was fixed or varied. (Figure 2B and 2C)

We tested utilizing MagVigen™-streptavidin magnetic nanoparticle beads for rare cell isolation in whole blood, because of the need in the clinic for CTC identification for cancer diagnostic and prognostic applications. Our procedure simply involved mixing of magnetic nanoparticle-antibody conjugates with the whole blood sample, then capture using an external magnet. For 4, 20 and 100 non-small cell lung cancer cells H1650 in whole blood, we were able to capture in average around 50% of the rare cell with high purity. A representative set of bright field and fluorescence images of captured H1650 cells were shown in Figure 2E and 2F. The images were taken for when 400k cells were spiked into whole blood. Basically, for every cells observed under bright field, there was a corresponding cell in green color from the CFSE pre-staining observed under the fluorescent field, demonstrating high purity of 100% for the isolated cancer cell populations in whole blood with our magnetic nanoparticle beads.

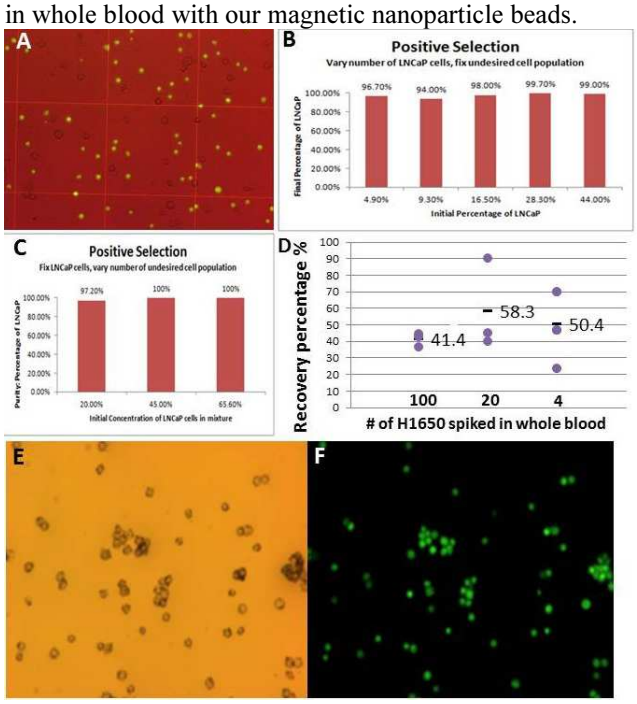

Figure 2. MagVigen™ magnetic nanoparticles enable high purity cell separation. A. An image showing the mixture of LNCaP and Oc1-ly8 (stained with CFSE, green fluorescence) cells before magnetic isolation. B and C. Positive selection of LNCaP cells using MagVigen™-streptavidin pre-conjugated with EpCAM-biotin showing high purity of selected cells over a wide range of mixing

ratios with either the number of Oc1-ly8 or LNCaP fixed. D. Small number of CTC separation in whole blood samples. Magnetic nanoparticle beads were directly mixed with whole blood. Magnetic separation provided 50% recovery yield of rare cells. E and F. Bright field (E) and fluorescent (F) images of magnetically separated H1650 cells in whole blood samples. 100% purity was observed.

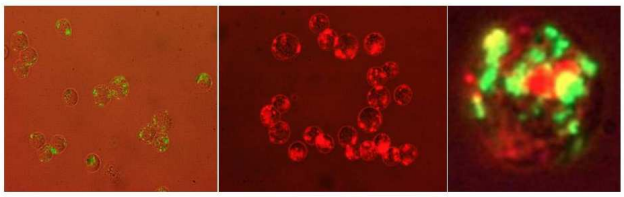

Figure 3. Concurrent magnetic separation and fluorescent labeling of human breast cancer cells MCF-7 using MyQuVigen™-535 nm-anti EpCAM conjugates (left panel), MyQuVigen™-615 nm-anti EpCAM conjugates (middle panel), and simultaneous multiplexed labeling of surface EGFR, EpCAM and Her2 markers with MyQuVigen-535 nm-anti EGFR antibody conjugates, MyQuVigen-585 nm-anti EpCAM conjugates and MyQuVigen-615 nm-anti Her2 conjugates, respectively.

The NID™ platform enables incorporation of multiple functional components within one single nanoparticle. For example, it could be utilized to make multifunctional nanoparticles with both magnetic and fluorescent properties. The magnetic property allows magnetic separation and magnetic resonance imaging when such need arises for *in vivo* imaging and tracking. The fluorescent material provides a detectable fluorescent signal which is widely used in many biological and medical labs. When using the fluorescent magnetic nanoparticles for cell separation, the magnetically captured cells are concurrently stained with a fluorescent signal for downstream fluorescent imaging or counting. Saving one additional cell staining step could save experimental time and reagent use; it is also better for cell viability with reduced processing steps. In addition, fluorescent signals could be directly applied to identify different cell types by tagging specific cell surface markers, enabling multiplexed cell identification. This could be extremely useful for more advanced cancer diagnostic and prognostic applications, where analysis of multiple surface markers is required for cancer staging and metastasis evaluation. Figure 3 shows the magnetically isolated cancer cells with bright and stable fluorescent signal when our MyQuVigen™ fluorescent magnetic nanoparticles are used. The left and middle panel of Figure 3 shows breast cancer cells MCF-7 specifically tagged with MyQuVigen™-535 nm emission, and MyQuVigen™-615 nm emission. On the right panel, MyQuVigen™-535 nm, MyQuVigen™-585 nm and MyQuVigen™-615 nm were used to label three respective surface markers, EGFR, EpCAM and Her2 on the target cell surface, respectively. These nanoparticles showed very high binding specificity of antibodies and cells. They expand the potential of magnetic cell separation and could provide a much better way to perform cell-based assays in an automatic, high-throughput and multiplexed format using both the highly responsive magnetic and highly bright and stable fluorescent properties of our MyQuVigen™ nanoparticles.

UTILIZING NANOPARTICLES BASED ON THE NID™ PLATFORM TO ENHANCE CANCER DRUG DELIVERY

Nanoparticle based anticancer drug delivery systems have been reported with many advantages over conventional chemotherapy such as targeted delivery, reduced drug resistance and side effects. [4, 5] However, there are only a few nanoparticle drug forms that have been FDA approved as injectables for humans, such as albumin based nanoparticles and lyposomes. These nanoparticles usually use the nanoparticle size effect for the intended applications. Many other advantageous aspects of using nanoparticle as drug carriers have not been fully exploited, for example, the possibility to precisely engineering nanoparticle size, shape, component material, surface property, drug loading to achieve optimal drug effect. In addition, nanoparticles represent a perfect platform to deliver multifunctional treatment solutions. The so called theranostics is one of the examples, where by incorporating both the imaging or signal generating materials and drugs into one functional nanoparticles, more advanced nanoparticle drugs could be developed and added to a clinician's toolbox. Presently, there are no drugs combining both diagnostic and therapeutic functions available in the clinic, more research and commercialization activities with necessary financial supports for developing more effective, practical and multifunctional nanoparticles are definitely needed to harness the power of nanotechnology to improve routine clinical practice.

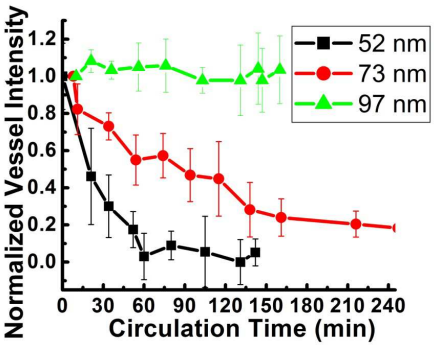

Figure 4. Tunabe pharmacokinetics of nanoparticles formed using the NID™ platform. With well controlled surface property, the circulating half life of nanoparticles is tunable by controlling the size of the nanoparticles. Optimal circulating half life is observed for the 97 nm nanoparticles. The sizes of the nanoparticles were obtained using DLS. The nanoparticle vessel intensity was measured through the fluorescence of the nanoparticles using an intravital microscope.

Our NID™ platform could generate the best-in-class nanoparticles for drug delivery applications. Our nanoparticle drug delivery platform is based on all clinically validated component materials. Nanoparticle size, shape, surface, and drug loading property could all be tuned to achieve optimal drug pharmacokinetics, biodistribution, cancer targeting and treatment effect. In addition, the intrinsic signal generating property of the component material, for example, the superparamagnetic property of the iron oxide components suitable for magentic resonance imaging, enables simultaneous diagnosis, treatment, and follow up evaluation from the same nanoparticle-drug.

Over the last two decades, much knowledge has been gained in various aspects governing the *in vivo* behavior of a nanoparticle. For example, adding PEG molecules on nanoparticle surface could extend the circulating life of nanoparticles and help to alleviate their sequestration by the body's immune system. However, it is very challenging to generate nanoparticles with optimal surface property. The need of high surface binding specificity is widely accepted and could be relatively clearly evaluated through well designed experiments. It is much more challenging to understand the surface property based on more sutle molecular balance that are difficult to quantity, such as the effect of surface charge, hydrophobicity/hydrophilicity, and distribution of surface charge and functional groups among the background molecules, not to mention many synergistic effects from multiple factors. Because of the complexity of living subjects, it is often necessary to guide the nanoparticle development through well designed *in vivo* experiments. By directly engineering the nanoparticle formulation based on its *in vivo* behavior, becautiful correlation between engineering the nanoparticle property and the corresponding tuning of their *in vivo* behavior could be observed. Using nanoparticles with the same surface property that has been developed through the balance of surface charge, hydrophobicity/hydrophilicity, and distribution of surface charge and functional groups among the background molecules, we could precisely tune the nanoparticle pharmakokinetics by simply adjusting the size of nanoparticles. As shown in Figure 4, the circulating half life of nanoparticles following intravineous injection could be tuned from less than 15 min to extremely long time. This property makes it possible to utilze our NID™ nanoparticle platform to tune the pharmaokinetics of incorporated drugs for optimized drug delivery.

The long circulating nanoparticles demonstrate extremely strong and extended retention in the tumor region. As shown in Figure 5, over 30 days tumor region retention was observed after a single dose of nanoparticle injection. Most commercial cancer imaging reagents including those in nanoparticle forms could only retain for a few days in the tumor region. Such extremely enhanced tumor permeability and retention effect represent one of the significant advantages of nanoparticles generated through

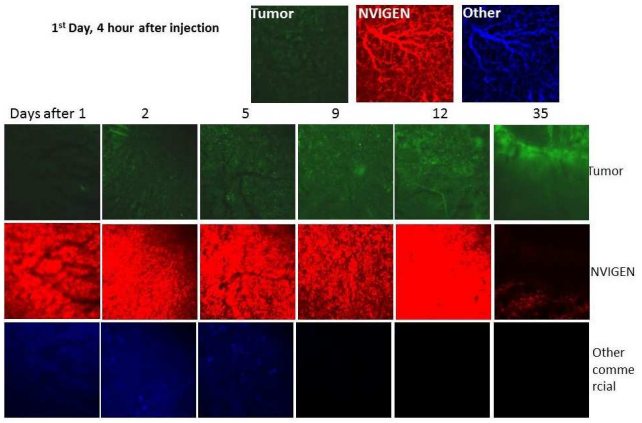

Figure 5. Extremely strong accumulation and long retention of nanoparticles in tumor region. The red signals are from the fluroescence of our nanoparticles generated through the NID™ platform. The green color shows the tumor cells which are transfected with EGFP. The blue signal is from commercial polymeric nanosized nanoparticles. The commercial long blood circulating nanoparticles could only stay in the tumor region for up to 5 days, while our NID™ generated nanoparticles demonstrated extremely strong accumulation in the tumor region with much extended retention effect for over one month.

our NID™ platform. Furthermore, the capability to precisely tune nanoparticle behavior *in vivo* and the intrinsic signal generating properties of the carrier materails make it highly promisimg to generate a next generation of nanoparticle drugs based on the NID™ platform with enhanced properties for cancer diagnosis and treatment.

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